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Cortisol Responses to Perceived Stress

in Depressed Individuals and Healthy Controls

by

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Abstract

Title of Thesis: The Effect of Depression on Cortisol Reactivity to Perceived Stress

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Major Depressive Disorder (MDD) is the most common psychological disorder in the United States. This disorder places people at increased risk for cardiovascular illness and mortality. Little is known, however, about the mechanisms that explain the relationship between depression and cardiovascular disease. One possible pathway includes exaggerated responsivity to challenge in depressed individuals. The hypothesis of this investigation is that depressed individuals demonstrate elevated neurohormonal and negative mood responses to mental and physical challenges as compared to non-depressed controls.

This investigation examined physiologic and self-reported stress responses in depressed and non-depressed individuals. Specifically, increases in salivary cortisol were examined in during laboratory-based mental challenge tasks and during daily life activities. The general hypothesis of this thesis was that participants with MDD (those scoring greater than 17 on the Hamilton Rating Scale for Depression) would show increased salivary cortisol responses to exogenous stimuli that increase levels of perceived stress as compared to non-depressed individuals in multiple settings. Salivary cortisol reactivity to mental challenge tasks (mental arithmetic and anger recall) and daily life stressors (using ambulatory mood and cortisol monitoring) were assessed in 14 depressed and 16 non-depressed control participants.

It was hypothesized that the relationship between depression and elevated levels of cortisol established in previous studies would be mediated by heightened levels of perceived stress in depressed individuals. This study did not find that depressed individuals demonstrated uniformly elevated cortisol levels as compared to non-depressed controls; in fact, blunted cortisol responses were observed among depressed participants in the ambulatory setting. In the laboratory, both depressed and control participants displayed a decrease in salivary cortisol levels in response to stressors and there was no significant difference between groups in change over time, suggesting that the effect of perceived stress on cortisol levels is not greater in depressed participants than in control participants. This observation may be explained by the relatively high baseline cortisol levels observed prior to the mental challenge task and by the time of day during which the measurements were taken. Overall, the role of perceived stress in laboratory and ambulatory salivary cortisol increases could not be confirmed in the present study.

Table of Contents

List of Tables

List of Figures

Introduction

Major Depressive Disorder (MDD) is associated with poor quality of life, increased health care costs related to health systems utilizations, and elevated risks of morbidity and mortality resulting from a broad range of diseases (Johnson, Weissman, & Klerman, 1992). Psychological distress plays a role in various stages of depression. Evidence indicates that stressful life events may precede depressive episodes; the sequelae of depression may elevate the perceived severity of distressing life events, and stressful life circumstances may contribute to the maintenance of depression (Kessler, 1997.) This thesis examines the role of perceived stress in the altered neurohormonal correlates of depression, particularly elevated levels of cortisol reflecting dysregulation of the hypothalamic pituitary adrenal axis.

The Hypothalamic Pituitary Adrenal (HPA) axis is one physiologic system through which depression may affect health. Individuals with depressive disorders show alteration in the function of the neuroendocrine axis compared to healthy controls (Brunner et al., 2002; Chrousos & Gold, 1992; Hjemdahl, 2002; Smith et al, 2005.) A specific aspect of altered neuroendocrine function attributed to depression is increased cortisol production. Activation of the hypothalamus causes the release of Corticotropin Releasing Factor, which stimulates the anterior pituitary to release Adrenocorticotrophic Hormone (ACTH) and subsequent activation of the adrenal cortex. This process results in elevated cortisol levels throughout the circulation. Individuals with MDD may also demonstrate impairment of the negative cortisol feedback loop and hypertrophy of the adrenal gland (Halbreich, Asnis, Shindledecker, Zumoff, & Nathan, 1985; Pfohl, Sherman, Schlechte, & Winokur, 1985.)

The increased activity of the adrenal cortex observed in depressed individuals is similar to the acute stress response. In individuals with typical MDD, activation of the HPA axis in response to stress in addition to elevated baseline levels of cortisol may result in exaggerated levels of cortisol. The additional impairment of the negative cortisol feedback loop may cause regulatory mechanisms to fail, such acute stressors can cause sustained cortisol activity among individuals with MDD rather than short-term HPA axis activation (Strohle & Holsboer, 2003.) These increased responses to perceived stressors may play an important role in the relationship between MDD and increased mortality and morbidity.

A series of physiological reactions occur that improve cardiovascular and respiratory function, increase energy mobilization, and decrease gastro-intestinal, reproductive, and inflammatory responses when an individual perceives a situation as “stressful” (Guyton & Hall, 2000.) Over time, repeated exposure to stressful experiences can have negative effects on the reactivity of physiologic systems. Thus repeated, or chronic, stress experiences create a condition referred to as allostatic load, in which the physiologic systems involved in the stress response become overly taxed (Sterling and Eyer, 1988.)

Investigations by Cannon, Selye, and Mason have resulted in the treatment of “stress” as a significant scientific and methodological topic. Difficulties in defining the multiple variables involved in this concept spurred Lazarus and Folkman (1984) to move away from the interplay of external stimuli and internal physiological processes that had been Selye’s interest, and instead to focus on specific cognitive processes mediating the physiological response to exogenous stressors. Today, modern definitions of stress emphasize both physiological responses and the individual evaluative processes involved in stress.

The present investigation will focus on physiologic and self-reported stress responses in depressed and non-depressed individuals. Specifically, salivary cortisol responses to self-reported perceived stress will be examined in laboratory and daily life settings. The general hypothesis of this thesis is that individuals with MDD will show increased salivary cortisol responses to exogenous stimuli that increase levels of perceived stress as compared to non-depressed individuals in multiple settings. The following sections will review the impacts of depression and perceived stress on physiologic and emotional well-being, and describe the investigation conducted to examine the interaction of these two effects. The methods and results of the research will be presented, followed by a discussion of the research findings.

Background

I. A Current Definition of Depression

The Diagnostic and Statistical Manual of Mental Disorders (DSM IV-TR) defines the criteria used to diagnose depression (APA, 2000.) Major Depressive Disorder (MDD) is diagnosed when an individual demonstrates five of the following nine symptoms: depressed mood, loss of interest in previously pleasurable activities, increases or decreases in sleep, changes in weight or appetite, fatigue, psychomotor agitation or retardation, feelings of guilt, decreased ability to concentrate, and suicidal ideation and/or attempts. The five present symptoms must include depressed mood and/or loss of interest, must occur for longer than 2 consecutive weeks, and must represent a significant source of distress or impairment.

The lifetime prevalence of MDD in U.S. adults is estimated at 17.1% (Kessler et al., 1994.) A diagnosis of MDD has been associated with increased use of general health care services, increased utilization of emergency room mental health services, lost time in the workplace, impairment of overall physical and mental health, and increased suicidality (Johnson, Weissman, & Klerman, 1992.) The cost of these health impacts of MDD, including provision of care and lost time, has been estimated at $83.1 billion per year in the U.S. alone (Greenberg et al., 2003.)

The prevalence of MDD, and the impact of the illness on physical health, is more prominent in individuals with comorbid medical conditions (Ormel et al., 1994; Spitzer et al., 1994.) The comorbidity of medical illness and MDD has been associated with increased disability, increased impairment of daily functioning, and poorer overall quality of life (Ormel et al., 1994; Spitzer et al., 1994.) Additionally, patients with MDD demonstrate increased rates of heart failure (Ramasubbu & Patten, 2003), renal failure

(Kimmel et al., 2000), cancer (Valente & Saunders, 1997), and total mortality (Wulsin, Vaillant, & Wells, 1999.)

Negative mood is also more prominent in individuals with MDD. In their diathesis-stress model, Kovacs and Beck (1978) propose that depressive symptoms manifest when the cumulative stressors in an individual's life exceed one's personal vulnerability threshold. It has been shown that accumulated stress, in the form of major life events, precedes the onset of depressive symptoms (Hooley, Orley, & Teasdale, 1986; Kessler, 1997.) In addition to these major events, recent studies have also shown that individuals with increased sensitivity to minor life events, or "hassles", are also vulnerable to depression (Malla, Cortese, Shaw, & Ginsberg, 1990.) This relationship has been demonstrated in individuals with a current diagnosis of MDD who show increased emotional sensitivity and self-reported negative mood responses to stressors in daily life as compared to non-depressed individuals (Myin-Germeys, Van Os, Schwartz, Stone, & Delespaul, 2001; Myin-Germeys et al., 2003.) The present study will evaluate the role of psychological distress as a mediator in the relationship between depression and cortisol levels in ambulatory and laboratory settings.

II. The Impact of Depression on Physical Health

One avenue by which MDD can cause negative health outcomes is altered neuroendocrine function. In individuals with MDD, altered HPA axis function is associated with increased secretion of cortisol from the adrenal cortex. Individuals with MDD may also demonstrate impairment of the negative cortisol feedback loop and hypertrophy of the adrenal gland. The most common HPA related correlate of MDD is elevated cortisol secretion, which occurs in approximately 50% of individuals diagnosed with MDD (Halbreich, Asnis, Shindledecker, Zumoff, & Nathan, 1985; Pfohl, Sherman, Schlechte, & Winokur, 1985.)

Increased activity of the adrenal cortex observed in MDD is similar to documented increases in individuals experiencing acute stress reactions. In individuals with typical MDD, the activation of the HPA axis in response to psychological distress in addition to elevated levels of cortisol resulting from depression may result in pathologically elevated levels of cortisol. The additional impairment of the negative cortisol feedback loop may cause regulatory mechanisms to fail, such that an acute stressor can cause sustained cortisol activity rather than short-term HPA axis activation in individuals with MDD (Strohle & Holsboer, 2003.) It is important to note that some individuals with MDD demonstrate “atypical” neurohormonal responses, in which cortisol secretion is decreased (Gold, Gabry, Yasuda, & Chrousos, 2002.) Hypocortisolemia depression has been associated with female sex, joint disease, smoking, and recurrent depression (Bremmer et al., 2006.)

III. The History and Development of “Stress” as a Scientific Construct

Despite its persistent and widespread use in biology and psychology, “stress” is a concept with a wide range of definitions. Before discussing the interrelationship between depression and psychological distress as related to HPA axis activation, a description of stress as a scientific construct will be provided. The term “stress” appears as early as 1914 in the biomedical literature, in a paper on emotion and psychoendocrine function by Walter Cannon. Cannon’s concept of stress included both physical and emotional stimuli, and focused on defining experiences which might overwhelm a person’s natural homeostatic mechanisms and thus alter the internal environment (Mason, 1975.) Cannon seems to have reflected the convention of the time in applying this stress concept, also referred to as "emotional stress" or "stress of excitement", to a variety of circumstances ranging from medical practice settings to social and industrial organization (Mason, 1975.)

Subsequent developments in stress research were substantially influenced by seminal animal studies by the Hungarian physiologist Hans Selye. By 1936 the use of the term "stress" was so common and imprecise that Selye initially avoided its use because of the "violently adverse public opinion" (Selye, 1956; Mason, 1975.) Selye's reports on the "general adaptation syndrome" postulated that some neural or humoral pathways were responsible for a nonspecific physiological response to "noxious" agents such as cold, heat, and exercise (Selye, 1936.) This response consisted of a three-stage biological process: the alarm reaction, the stage of resistance, and the stage of exhaustion (Selye, 1936.) Selye did not label his noxious agents as "stress" until 1946, when he described the general adaptation syndrome as a "defensive endocrine response" that "facilitates adaptation to stress" (Selye, 1946.) At this time, the concept of "stress" referred primarily to physical and emotional *stimuli*.

In subsequent work by Selye, "stress" was redefined as "systemic stress" to describe a condition within the organism that occurs as a *response* to "evocative agents." This proposition was revised in 1951 when Selye discussed "stress" as the *interaction* between the stimulus, or "*stressor",* and the response, "*stress*." His final definition was not put forth until the 1955/1956 volume of the Annual Reports on Stress was published. In this publication, Selye attested that "stress is fundamentally a physiological *response*" defined as "the sum of all nonspecific changes caused by function or damage" (as cited by Mason, 1975.) In 1975 Selye expanded the possible stimuli included in his definition from an already wide variety of agents to “any demand” made upon the body, with agreeable demands causing “eustress” and disagreeable demands causing “distress” (Selye, 1975.) This new concept of “stress” extended the formulation proposed in the 1950s, referring not to the external stimulus but to the body’s internal systemic response.

Selye’s argument for the “general adaptation syndrome” emphasized one standard physiological response to all types of stress. This “nonspecificity” approach was countered by John Mason (1975), who examined research studies for evidence of differing stress responses. Mason criticized experimental designs that allowed psychological factors such as “emotional disturbance, discomfort, or even pain” to interfere with the measurement of physiological stressors such as “heat, cold, exercise, trauma, and so on.” Mason advocated research that reduced or carefully measured the “interfering effects” of psychological reactions to experimental settings, and cited reduced corticosteroid and adrenal hormone reactions to physical stressors when factors such as novelty, temperature, and the discomfort of laboratory animals were minimized. Mason’s argument for the consideration of psychological processes that contribute to, and possibly even change, physiological stress responses was an important addition to the concept of “stress.”

Although the original investigations by Cannon, Selye and Mason contributed significantly to the research on biological consequences to environmental challenges, the term “stress” has continued to raise significant scientific and methodological concerns. Ader (1981) argued against any use of the word “stress”, because individual perceptions of stimuli and any consequent responses occur within a larger context. This context is characterized by an individual’s genetic and experiential background, social and cultural context, and coping style within a preexisting psychophysiological state that are unique to the individual. The presence of these variables during a threatening experience creates a complex situation that cannot be defined as uniformly detrimental or beneficial. Because the word "stress" has come to imply a detrimental stimulus or effect, it should arguably not be used to describe such a complicated phenomenon.

The difficulties in defining the multiple variables addressed by Ader led many to search for a more refined concept of "stress." In the field of psychology, Lazarus and Folkman (1984) built on the work of Grinker and Spiegel (1945), Arnold (1960), and others in addressing the importance of the individual evaluative processes involved in stress. This approach moved away from the interplay of external stimuli and internal physiological processes that had been Selye's interest, and instead narrowed the focus to specific cognitive processes mediating the physiological response to exogenous stressors. This development in the conceptualization of stress has added to the understanding of the inter-relationship between stress and the onset and persistence of depression. In the present study, individual responses to the perceived distress associated with a provocative psychological stressor (anger recall and mental arithmetic) will be examined. In addition, levels of retrospectively reported distress and ambulatory assessments of perceived stress will be obtained. These three domains (retrospective report, ambulatory assessment, and distress responses) will provide unique opportunities to evaluate the role of stress as a mediator between depression and HPA dysregulation.

IV. The Interaction of Stress and Depression

As previously discussed, both the perception of stress and MDD can cause alterations in neuroendocrine function. In individuals with MDD, certain aspects of the stress response may become escalated. The present study will focus on changes in HPA axis stress response associated with MDD. For the purposes of this study, stress is operationally defined as "a state of physical or psychological strain which imposes demands for adjustment upon the individual" (Corsini, 2002.) Psychological stress will be assessed in three domains: (1) retrospectively reported perceived stress; (2) distress as observed during ambulatory monitoring; and (3) distress as provoked in the laboratory setting using mental challenges. A detailed discussion of the history and conceptualization of stress will be presented in the following section.

Differential responses between depressed and non-depressed individuals exist following exposure to emotional and mental challenges. The purposeful recall of a distressing event and a mental arithmetic task will be used in this study to examine emotional and mental stress. In healthy individuals, HPA activation as well as self-reported negative mood has been associated with emotional and mental stress (Oltras, Mora, & Vives, 1987.) Depressed individuals have demonstrated the same responses at an exaggerated level (Feldman et al., 1999.) The present study will examine laboratory-based reactivity to mental and physical challenge, as well as ambulatory assessment of distress in depressed and non-depressed individuals.

V. A Current Definition of Stress and Relevance to Depression

In this thesis, stress will be examined using the model proposed by Lazarus and Folkman. In this model a potentially distressing stimulus is referred to as the stressor, and the stress response is comprised of two separate components, referred to here as appraisal and affect. The Lazarus and Folkman model of stress describes the cognitive process by which an individual evaluates the environment and makes decisions about appropriate behaviors (Monroe & Kelley, 1997.) This evaluative process is referred to as appraisal, and it occurs in a primary and a secondary stage. At each stage, depression may adversely affect the process of appraisal and result in increased perceived stress.

Primary appraisal involves evaluation of a specific environmental situation in relation to the individual's well-being. As outlined by Monroe and Kelley (1997), there are three types of primary appraisal; irrelevant, benign-positive, and stressful. Irrelevant and benign-positive situations do not present a threat to the individual and do not trigger a stress response. Situations appraised as stressful require some action on the part of the individual, and are categorized as harm/loss (harm or damage to the individual has occurred), threat (harm or damage may occur), or challenge (an opportunity for growth or gain has been presented.)

Secondary appraisal is the cognitive process through which an individual evaluates his or her capabilities for dealing with the situation. This evaluation includes an examination of available coping strategies, an evaluation of the likely success of a given strategy, and an estimation of the individual's ability to actually perform the strategy. The negative thought patterns associated with MDD may impact this evaluation and result in altered appraisal of events and coping skills, resulting in increased levels of perceived stress in depressed individuals. Depressed individuals have been shown to appraise events as more unpleasant and stressful than non-depressed control participants (Beck, Rush, Shaw, & Emery, 1979), and to demonstrate less active coping in stressful settings (Gunthert, Cohen, & Butler, 2005.) In this way, the complex interactions between the individual and the environment described by Lazarus and Folkman are further complicated by negative appraisal, unpleasant mood, and less active coping associated with MDD.

VI. Physiologic Measures of the Impact of Stress

A. Acute and Chronic Exposure to Stress

Short term, or acute, stressors result in physiological reactions that improve cardiovascular and respiratory function, increase energy mobilization, and decrease gastro-intestinal, reproductive, and inflammatory responses (Guyton & Hall, 2000.) This reaction is often referred to as the "fight or flight" response, because it prepares the body to confront potentially dangerous environmental hazards. Sterling and Eyer (1988) labeled the physiological process through which the body responds to a stressor and then returns to normal function allostasis.

Over time, repeated exposure to stressful experiences can have negative effects on the reactivity of the allostatic system. These repeated, or chronic, stress experiences purportedly create a condition referred to as allostatic load, in which the physiologic systems involved in the stress response become overly taxed (Sterling and Eyer, 1988.) Allostatic load can describe situations in which exposure to chronic stressors causes over-reaction, under-reaction, or a failure to return to normal operating levels. Allostatic load influences multiple biologic systems and is thought to be the primary mechanism through which chronic stress affects physiological health (McEwan, 1998.)

B. Neurohormonal Responses to Stress Exposure

The Hypothalamic Pituitary Adrenal (HPA) axis is one specific biologic system through which perceived stress may affect health. Individuals experiencing chronic distress show alteration in the function of the neuroendocrine axis compared to healthy controls (Brunner et al., 2002; Hjemdahl, 2002; Smith et al, 2005.) This alteration can include increased cortisol, catecholamine, and androgen production as well as changes in cardiac activity and inflammatory factors (Brunner et al., 2002; Hjemdahl, 2002.)

Continuously elevated neuroendocrine function, which is a form of allostatic load, may be causally related to the development of a wide range of disease states (Hjemdahl, 2002.)

One aspect of altered neuroendocrine function attributed to the perception of stress is increased cortisol production. Activation of the hypothalamus causes the release of Corticotropin Releasing Factor, which stimulates the anterior pituitary to release Adrenocorticotrophic Hormone (ACTH) and in turn activate the adrenal cortex. This sequence during perceived stress results in elevated levels of cortisol, a corticosteroid hormone, throughout the body.

The HPA axis is one of several neuroendocrine systems that display circadian changes. Daily changes in HPA axis activity, including cortisol production, are attached to the sleep-wake cycle such that the highest levels occur shortly after waking and the lowest levels occur during the early parts of the sleep cycle. Following the sharp morning rise, cortisol levels drop sharply and then demonstrate a gradual decline throughout the day. This rhythm has been demonstrated in both sexes and across a wide span of ages (Ranjit et al., 2005).

Research demonstrates that the magnitude of the morning rise in cortisol is associated with the level of perceived stress present in an individual's life (Pruessner et al., 1999). Basal activity and acute stress reactivity (cortisol levels following the onset of a stressor and the recovery phase as levels return to baseline following the offset of a stressor) have also been shown to vary in the presence of perceived stress (Burke et al., 2005). As such, measurement of cortisol daily rhythm and morning rise can provide a means to examine the impact of stressors on individual biological function (Ranjit et al., 2005).

Cortisol levels can be measured in an individual's urine, blood, and saliva. There are challenges associated with all three of these measurement types. Measuring cortisol in an individual's urine does not allow for the assessment of rapid changes in cortisol levels (Gozansky, Lynn, Laudenslauger & Kohrt, 2005), instead relying on cortisol metabolites excreted from the body over time. Measurement of cortisol in the blood requires analysis of serum cortisol, which is either bound or free (Kudielka & Kirschbaum, 2005.) Over 80% of the cortisol produced in the body is bound to globulins such as cortisol-binding-globulin (CBG) for transport through the bloodstream. Levels of CBG vary between individuals and are not typically considered in the measurement of serum cortisol (Dhillo et al., 2002.) This variation in CBG creates a problem in the measurement of overall cortisol production unless between-person differences in CBG are measured and corrected for (Dhillo et al., 2002.) Radioimmuno assays are available to assess free cortisol. Salivary measures analyze biologically active cortisol that is not bound (Kudielka & Kirschbaum, 2005.) This measure is less invasive than plasma collection and relatively inexpensive, but also presents a measurement problem. In a study by Broderick, Arnold, Kudielka, and Kirschbaum, study participants providing ambulatory samples for analysis were found to show relatively low compliance with sampling protocol (2004.) Thus salivary samples present the best option for cortisol measurements, but should be monitored for participant compliance. In the present study, we will use both ambulatory and laboratory cortisol measures to rule out potential biases related to participant compliance.

Summary & Hypotheses

Among individuals with depressive mood disorders, HPA responses may be disproportionately exaggerated during resting conditions, and potentially even more in response to psychological challenges. These increased responses to exogenous stimuli that result in increased levels of perceived stress may play an important role in the relationship between MDD and increased mortality and morbidity. The purpose of this study is to examine the impact of MDD on salivary cortisol as measured during daily life activities and in the laboratory. In addition, the role of perceived stress in the relationship between MDD and HPA dysregulation will be examined. The specific aims and hypotheses for this study are:

Aim 1: Examine overall pattern of diurnal cortisol levels in depressed and control participants. Ambulatory cortisol measures were collected at various time points throughout the day. Increases in the waking response of cortisol levels will be used as primary end-points in the comparison of depressed versus non-depressed individuals. The examination of the waking response is based on previous research establishing waking as an endogenous stimulation of the HPA axis. Specifically, morning cortisol increases have been associated with prolonged psychological distress (Schulz, Kirschbaum, Pruessner, & Hellhammer, 1998.) It is expected that depressed individuals experience heightened levels of perceived stress which will be reflected in elevated levels of cortisol at waking. We will also examine cortisol levels at other time points for exploratory purposes.

Hypothesis 1: Depressed participants will show greater increases from bedtime to waking salivary cortisol levels than will non-depressed controls.

Aim 2: Examine the reactivity of cortisol levels to acute laboratory stressors in depressed and control participants. The laboratory cortisol measures collected in this study include baseline, immediately following an acute mental challenge task, and at 60-minute recovery. Baseline cortisol levels can be compared to levels following mental challenge tasks to establish the magnitude of cortisol responses to stress in depressed and non-depressed controls.

Hypothesis 2: Depressed participants will show a greater cortisol response to laboratory challenges than will non-depressed controls.

Aim 3: Examine the role of perceived stress in laboratory and ambulatory salivary cortisol levels. The present study collected data on three domains of perceived stress: (a) retrospective self-report using the Perceived Stress Scale examining distress over the past month; (b) ambulatory self report of distress levels during 24 hours of monitoring; and (c) acute perceived stress response to a laboratory stressor. The general hypothesis underlying this aim is that differences in cortisol levels in depressed versus non-depressed individuals are mediated by elevated levels of perceived stress. Specifically, it is hypothesized that:

H3A – Elevated levels of retrospectively reported perceived distress (Based on the PSS) will be related to elevated baseline cortisol levels in the laboratory and ambulatory settings.

H3B – Elevated stress levels reported in ambulatory diaries will be related to elevated ambulatory cortisol levels and an increased cortisol response to awakening.

H3C – Elevated acute increases in perceived distress (assessed by Likert scores following mental challenge tasks) will be related to elevated cortisol levels in the laboratory setting.

All measures of perceived stress will be also be compared between depressed and control participants in order to examine whether depression is associated with elevated levels of retrospectively reported distress, ambulatory distress, and acute distress responses to mental challenge tasks.

Research Design and Methods

I. Overview

Psychological and physiological measures were obtained during one continuous visit to the Human Performance Laboratory at the Uniformed Services University of the Health Sciences. Ambulatory measures began after the laboratory visit and were conducted for approximately 24 hours by participants during normal daily activities.

II. Participants

Participants were recruited as part of a larger study examining multiple biochemical responses to mental and physical challenge (14 depressed participant and 16 control participants.) Of the total study participants (N=30), complete laboratory cortisol responses were available in 14 depressed and 15 control participants, and complete ambulatory saliva samples were available for 12 depressed and 9 control participants.

Depressed participants: Participants were defined as depressed using the Hamilton Rating Scale for Depression (HRSD) standardized interview, with scores greater than 17 indicative of depression (Hamilton, 1967.) Potential participants were excluded if any of the following criteria were met: (1) age < 18; (2) positive history of cardiovascular disease including hypertension; (3) use of antihypertensive, immunomodulatory, or anti-inflammatory medications other than aspirin; (5) history of bipolar disorder or previous treatment for psychosis; (6) current treatment for a mental disorder other than anxiety or depression; (7) active suicidal plans; (8) refusal of informed consent. All depressed participants were under psychiatric or psychological treatment, or were provided referrals for treatment. Structured interviews were conducted by a licensed clinical psychologist.

Healthy controls: Absence of depression was documented using the HRSD; with scores less than 7 indicative of non-depressed status (Hamilton, 1967.) Healthy controls were matched to depressed participants on smoking status, gender, and age in the larger study. Exclusion criteria for controls were the same as for depressed participants, with criterion 6 modified such that control participants were excluded if they had a known history or current evidence of any mental disorder.

III. Procedures:

Participants were recruited from the Washington, D.C. area. Interviews and data collection were conducted at the Uniformed Services University of the Health Sciences in the Human Performance Laboratory. All tests were performed between 1 and 4 PM to control for diurnal effects on target physiological and behavioral parameters. Participants first provided written informed consent, after which the HRSD was administered to determine depression status, and questionnaires to evaluate demographic information and baseline psychosocial variables. An indwelling catheter was inserted and the participant began a series of mental and physical challenge exercises. At the completion of the laboratory tasks, participants were provided with the materials for 24 hours of ambulatory monitoring of mental and physical measures.

Ambulatory Procedure

Ambulatory monitoring started following completion of the laboratory assessments (between 4 and 6 PM.) Participants used pocket-sized diaries to monitor mood and somatic symptoms during daily live events (Hedges, Krantz, Contrada, & Rozanski, 1990; Patterson et al., 1993; Krantz et al., 1996.) Salivary cortisol measures were provided at six intervals over a 24-hour period. Based in the findings of the MacArthur Report on Salivary Cortisol Measurement (2000), measurements were taken at each participant's natural waking time and at approximately 1, 4, 9, and 11 hours after waking. The diary and cortisol measures will be described in detail in the measures section of this paper.

Laboratory Mental Challenge Procedure

Laboratory mental challenge was used to evaluate cortisol and emotional responses in both depressed and healthy control participants. Because some physiological stress-response parameters require exposure times of greater than five minutes to generate detectable responses (Kunz-Ebrecht, Mohamed-Ali, Feldman, Kirschbaum, & Steptoe, 2003), participants completed two consecutive tasks for a total duration of 10 minutes.

The anger recall task involves a speech regarding a recent frustrating or anger-inducing event in the participant's life (Ironson et al., 1992; Kop et al., 2004.) This task elicits significant emotional and hemodynamic responses. Anger recall tasks were audiotaped for the purpose of content and quality review.

Participants then completed a five-minute mental arithmetic task. This task involves subtracting serial sevens from some four-digit number while being encouraged to work as quickly and as accurately as possible (Gottdiener et al., 1994; Kop et al., 2004.) This task elicits neurohormonal, hemodynamic, hemostatic, and among patients with coronary artery disease also ischemic responses (Blumenthal et al., 1995; Jern et al., 1991; Kop et al., 2004) that have been shown to be independent of talking alone (Rozanski et al., 1988.) The order of completion of the anger recall and mental arithmetic tasks was counterbalanced. The primary responses to these challenges relevant to the current study include perceived stress and salivary cortisol levels.

IV. Measures Obtained:

Psychological measures

As previously mentioned, depression was measured in this study using the HRSD. Depression was additionally assessed using the Beck Depression Inventory II (BDI-II) (Beck, Steer, & Brown, 1996.) This measure consists of 21 self-report items, with scores above 10 considered indicative of the presence of depression. The BDI-II has shown excellent reliability (Cronbach's alpha = 0.92-0.93.)

The Perceived Stress Scale (PSS) was used to evaluate retrospective levels of distress (Cohen, Kamarck, & Mermelstein, 1983.) The PSS focused on levels of distress experienced by participants in the week prior to the laboratory visit. The reliability of this measure is good (Cronbach alpha = 0.84-0.86) (Cohen et al., 1983.)
Levels of distress during ambulatory monitoring were obtained using a 5-point Likert scale which is provided in the Appendix. The Likert measure was 25-items long and measured "feelings that people may have", including a "stressed" item. In addition to the Likert scale, the short-form of the POMS (POMS-SF) was used. The POMS-SF is composed of 30 emotion labels that the participant is asked to rate on a 7-point Likert scale ranging from "not at all" to "extremely." Crohnbach's alpha for this measure

ranges from 0.85 to 0.97 (McNair, Lorr, & Droppleman, 1992.) In the present study, only the anger, fatigue, and vigor subscales of the POMS-SF were issued in order to keep the patient burden to a minimum. For the present analyses, we focused on the added item "stressed", which participants completed after 30 min. rest, immediately following mental challenge, and after 60 min. recovery.

<u>Physiological measure</u>

Laboratory cortisol samples were taken under the supervision of study staff. Participants provided samples prior to the mental challenge tasks in order to establish individual baseline levels. Samples were collected again after the onset of the stressor, band 60 minutes after the offset of the stressor (Kirschbaum and Hellhammer, 1989.) To provide salivary cortisol samples, participants placed a roll of cotton in their mouths, chewed it for approximately 30 seconds to allow saturation, and placed it in a plastic tube called a salivette (Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005.) Samples were refrigerated until shipment to the core laboratory. Assays were conducted as described previously in Polk, Cohen, Doyle, Skoner, and Kirschbaum (2005.) In brief, cortisol levels were determined by time-resolved immunoassay with flourometric end point detection. Intra- and inter-assay variabilities for this method average less than 12%.

Ambulatory cortisol samples were provided by participants over a 24-hour period following the laboratory visit. Participants were provided with six labeled salivettes and instructions on obtaining saliva samples in an ambulatory setting. Samples were provided at (1) 4PM and at (2) bedtime on the day of the lab visit , and at (3) each participant's natural waking time and (4,5,6) approximately 1, 4, 9 hours after waking on the day following the lab visit. Participants were instructed to record the specific time each sample was collected using the ambulatory diary. This collection protocol is on keeping with established study protocols designed to capture critical aspects of diurnal cortisol variations using as few measures as possible (Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005; Ranjit, Young, Raghunathan, & Kaplan, 2005.) Participants stored salivettes in a refrigerator until all samples were collected and returned by mail to the study staff.

Ambulatory diary measure

This study used a diary system validated in several previous studies for assessing daily activities and mood states in ambulatory settings (Patterson et al., 1993.) Adequate reliability and validity ratings for this measure in assessing levels of mental and physical activities have been demonstrated (Krantz et al., 1995.) The diary consists of a checklist of physical activities which participants rate as dichotomous variables (yes/no) and mood ratings which participants rate on a 5-point Likert scale. Participants were provided with a 55-page diary with directions for use after the completion of the lab portion of the study. They were instructed to complete a new diary page any time their activities changed during the day, and to record the time of each salivary cortisol sample provided. Participants generally began providing data at 4PM following the laboratory portion of the study. Recording intervals ranged from several minutes to several hours, and multiple physical activities were frequently recorded.

V. Statistics & Power Analysis

Hypothesis 1: Depressed participants will show greater increases from bedtime to waking salivary cortisol levels than will non-depressed controls.

Statistical Analysis: To examine the difference between bedtime and waking levels of salivary cortisol in depressed and control participants, a 2x2 mixed model ANOVA for repeated measures will be conducted.

Power analysis: Using the procedures for power estimation described by Cohen (Cohen, 1988), with 15 participants per group this study is able to detect between-group differences at a power of 0.80, and an alpha level of 0.05. This power analysis was computed using an effect size of 0.42 based on previous research examining ambulatory cortisol responses (Pruessner, Hellhammer, Kirschbaum, & Clemens, 1999; Polk, Cohen, Doyle, Skoner, & Kirschaum, 2005.)

Hypothesis 2: Depressed participants will show a greater cortisol response to laboratory challenges than will non-depressed controls.

Statistical Analysis: To compare salivary cortisol levels of depressed and control participants in the lab, a 2x3 mixed model ANOVA for repeated measures will be conducted.

Power analysis: Using the procedures for power estimation described by Cohen (Cohen, 1988), with 15 participants per group this study is able to detect between-group differences at a power of 0.80, and an alpha level of 0.05. This power analysis was computed using an effect size of 1.0 based on previous research examining cortisol responses in laboratory settings (Burke, Davis, Otte, & Mohr, 2005.)

Hypothesis 3: Elevated perceived stress scores will be related to elevated cortisol levels in both laboratory and ambulatory settings.

H3A – Elevated scores on the PSS will be related to elevated baseline cortisol levels in the laboratory and ambulatory settings.

H3B – Elevated self-reported stress will be related to elevated ambulatory cortisol levels.

H3C – Elevated Likert scores in response to acute lab responses will be related to acute cortisol responses in the laboratory setting.

Statistical Analysis: Initial exploratory analyses will be conducted using product-moment correlations to examine whether each individual domain of distress is related to cortisol levels. For those measures that are correlated, regression models will test the ability of multiple measures of perceived stress to predict salivary cortisol elevations in depressed and control participants.

Power analysis: The power estimation conducted for a regression model using 3 or more predictor variables with a power of 0.80 at an alpha level of 0.05 indicates that with a sample of 30 participants we will be able to detect an r of 0.48.

Results

I. Participant Characteristics

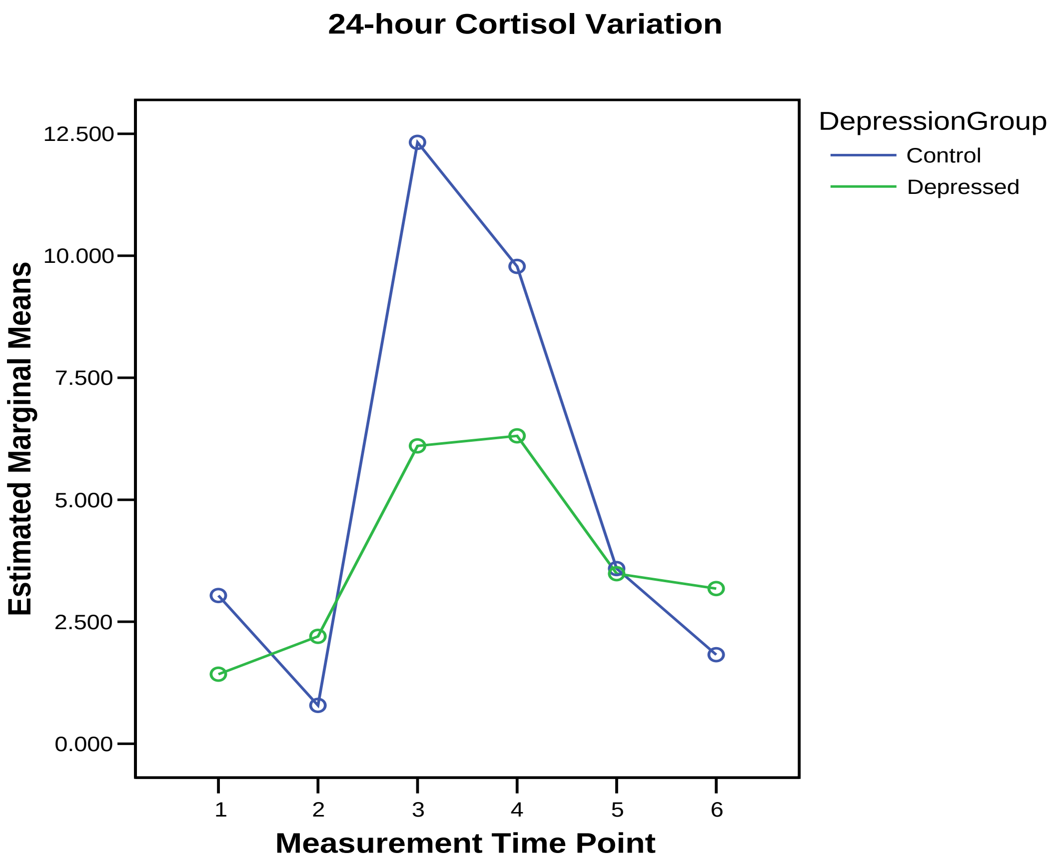
Analyses were based on a larger study included 14 participants with depression and 16 non-depressed controls (total N = 30) (Weinstein, 2007.) The groups did not differ on race ($p = 0.61$), marital status ($p=0.58$), years of education ($p=0.29$), employment status ($p=0.67$), or income ($p=0.54$.) Participants were successfully matched for age, gender, and BMI. Complete laboratory cortisol samples were available for 28 of the 30 participants (12 depressed, 16 control); 23 participants provided waking and after-waking ambulatory cortisol samples (12 depressed, 11 control); and 22 participants provided complete ambulatory diaries (11 depressed, 11 control.) In the subgroup that completed the ambulatory cortisol and diary measures, control participants were more educated than depressed participants. There were no other significant differences between depressed and non-depressed individuals in any of the other subgroups.

II: Hypothesis 1: Difference between ambulatory salivary cortisol levels in depressed versus control participants

Table 1. Salivary Cortisol at Bedtime and at Waking

	Control		Depressed	
	Bedtime n = 11	Waking n = 11	Bedtime n = 12	Waking n = 12
Salivary Cortisol (nmol/l)	0.77 (±0.66)	12.37 (±4.95)	1.87 (±4.16)	5.75 (±3.12)

A statistically significant main effect was observed, indicating an increase in salivary cortisol levels from bedtime to awakening (F_{time} (1, 21) = 45.66; $p < 0.01$.) There was also a significant main effect of group status (F_{group} (1, 21) = 7.92; $p = 0.01$), demonstrating lower ambulatory salivary cortisol levels in depressed versus control participants. The interaction term was also significant (F_{group} (1, 21) = 11.37, $p < 0.01$.) Examination of the interaction term by analyzing simple effects indicated that the groups did not differ significantly at bedtime ($t_{bedtime}$ (21) = -0.87; $p = 0.40$) or at waking (t_{waking} (22) = 1.58; $p = 0.13$.) Analysis of differential responses between depressed and control participants to waking showed that control participants showed a strong response ($t_{control}$ (10) = -8.34; $p < 0.01$), whereas depressed participants showed a somewhat lessened response ($t_{depressed}$ (11) = -2.18; $p = 0.05$.) This finding refutes the hypothesis that depressed individuals would demonstrate a greater salivary cortisol response to awakening as compared to control participants.



III. Hypothesis 2: Salivary cortisol response to laboratory mental challenge in depressed and control participants.

Salivary cortisol levels were measured at baseline, immediately following the laboratory challenge task, and at 60 minutes recovery.

Table 2. Salivary Cortisol Before, Immediately After, and 60 Minutes After the Mental Stress (MS) Challenge.

	Control N = 16			Depressed N = 12		
	Before MS	After MS	60 Minutes	Before MS	After MS	60 Minutes
Salivary Cortisol (nmol/l)	4.00 ±2.08	4.19 ±2.58	2.63 ±1.24	5.39 ±4.52	4.71 ±4.07	3.06 ±2.07

Significant changes in cortisol were observed for repeated measures over time (F_{time} (2, 25) = 9.57; $p < 0.001$). No main effect for depression status on cortisol levels was found (F (1, 26) = 0.66; p = 0.42.) The interaction term was non-significant ($F_{interaction}$ (1, 26) = 0.86; p = 0.36.) Consistently, there was no significant difference between groups in change over time (t_{change} (27) = -1.12; p = 0.27.) These findings suggest that the effect of mental challenge on cortisol levels is not greater in depressed participants than in control participants. Furthermore, the direction of change in cortisol did not support the hypothesis that acute mental challenge would increase cortisol levels, as the main effect was primarily attributable to the low recovery levels rather than elevated cortisol levels in response to mental challenge (Table 2.)

Table 3. Perceived Stress Before, Immediately After, and 60 Minutes After the Mental Stress (MS) Challenge

	Control N = 16			Depressed N = 14		
	Before MS	After MS	60 Minutes	Before MS	After MS	60 Minutes
Perceived Stress (Mean Level)	1.56	2.94	1.25	3.29	5.14	3.64

IV. Hypothesis 3: Relationships between perceived stress and cortisol levels in laboratory and ambulatory settings. Perceived stress measures taken in this study were the Perceived Stress Scale (one month retrospective recall), ambulatory ratings, and Likert ratings (for acute laboratory-based mental challenge response.)

Hypothesis 3A: Elevated levels of retrospectively reported perceived distress (based on the PSS) will be related to elevated baseline cortisol levels in the laboratory and ambulatory settings.

In order to examine the relationship between perceived stress and resting cortisol levels, PSS scores were correlated with baseline laboratory and pre-bedtime cortisol levels. Depressed individuals had higher PSS scores than controls (22.79 ± 8.40 vs. 7.50 ± 7.61 F = 27.38; $p < 0.01$.) However, neither laboratory ($r = 0.18$, $p = 0.35$) nor ambulatory ($r = 0.13$, $p = 0.57$) resting cortisol levels were significantly related to perceived stress scores. This was true in both depressed ($r_{laboratory} = 0.37$, $p = 0.22$; $r_{ambulatory} = -0.05$, $p = 0.88$) and control ($r_{laboratory} = -0.47$, $p = 0.07$; $r_{ambulatory} = 0.46$; $p = 0.15$) participants. The relationship between PSS scores and laboratory resting cortisol levels approached significance only in control participants, which is in contrast to the hypothesis that perceived stress influences elevated cortisol levels in depressed individuals. When statistically adjusting for PSS scores, the difference in ambulatory (beta = 0.01, $p = 0.96$) and laboratory baseline (beta = 0.08, $p = 0.79$) cortisol levels remained non-significant.

Hypothesis 3B: Elevated self-reported levels of ambulatory distress will be related to elevated ambulatory cortisol levels.

In order to examine the relationship between self-reported distress during daily life activities and ambulatory cortisol levels, diary ratings of perceived stress were correlated with cortisol morning increases and daily averages. Perceived stress diary ratings were categorized by total time spent at each Likert level, such that each participant received a total number of minutes spent at stress levels 1 through 5 for the two day monitoring period. This method also accounted for time spent sleeping and time without accurate record in the diary. Thus, each participant's was rated on the number of minutes out of the total 1440 minutes in the 24 hour period spent at each Likert level.

Initial examination of group status and time spent at each ambulatory distress level revealed that depression was associated with less time spent in low-level ambulatory distress, or level 1 ($F = 6.34$; $p = 0.02$.) As shown in Table 4, no other differences were found in ambulatory measures of distress among depressed versus control participants. Daily cortisol averages also demonstrated a significant correlation with perceived stress level 2 in control participants. There were no significant relationships with any other levels of perceived stress.

Table 4. Ambulatory Distress and Depression Group Status

	Relationship to Depression Group Status	
	F	p
Distress Level		
1	6.34	0.02*
2	1.42	0.25
3	3.51	0.77
4	0.05	0.83
5	1.96	0.18
Sleep	0.83	0.37
Missing	6.68	0.02

The only significant relationship between perceived stress diary ratings and morning increases in cortisol was in control participants who reported their stress levels at 2 out of 5 on the Likert scale (Table 5), and this association was in the opposite direction from that hypothesized. This finding does not support the hypothesis that perceived stress would influence the cortisol response to waking as a stressor in depressed participants.

Table 5. Diary Stress Levels and Cortisol Awakening Responses

	Total N= 22		Control N=11		Depressed N= 11	
	r	p	r	p	r	p
Distress Level						
1	0.06	0.80	-0.44	0.18	0.32	0.37
2	0.08	0.73	0.78	<0.01*	0.21	0.55
3	-0.01	0.95	0.48	0.14	0.24	0.51
4	-0.14	0.53	0.05	0.90	-0.59	0.07
5	-0.27	0.23	none	none	0.01	0.97
Sleep	-0.08	0.73	-0.11	0.75	-0.16	0.64
Missing	0.06	0.81	0.23	0.50	0.26	0.34

In addition to the awakening response, ambulatory distress ratings were also correlated with average daily cortisol levels. The six measures of salivary cortisol were averaged for each individual and compared to the time spent at each level of stress. As shown in Table 6, there was a somewhat significant relationship between perceived stress diary ratings and cortisol daily averages in control participants who reported their stress levels at 2 out of 5 on the Likert scale. This finding does not support the hypothesis that perceived stress would influence daily average cortisol levels in depressed participants.

Table 6. Diary Stress Levels and Cortisol Daily Averages

	Total N = 22	Control N = 11		Depressed N = 11	
	mean (s.d.)	r	p	r	p
Distress Level					
1	461.45 (357.04)	-0.37	0.29	-0.14	0.73
2	122.68 (148.85)	0.66	0.04*	-0.00	0.99
3	123.18 (159.19)	0.47	0.17	0.32	0.39
4	62.00 (131.78)	0.08	0.84	0.38	0.31
5	4.09 (14.03)	none	none	-0.37	0.32
Sleep	461.45 (357.04)	-0.37	0.29	-0.12	0.76
Missing	122.68 (148.85)	0.34	0.34	-0.78	0.85

Hypothesis3C: Likert score elevations in response to acute lab stressors will be related to acute cortisol responses in the laboratory setting.

Perceived stress levels were measured at baseline, immediately following the laboratory stressor, and at 60 minutes recovery. Participants completed a self-report 7-point Likert scale describing how "stressed" they felt.

In order to examine the overall differences in perceived stress between depressed and control participants, paired t-tests were used. There were significant differences in perceived stress from baseline to the onset of the stressor, and from the onset to 60-minutes after the offset in the depressed and the control groups, indicating that the mental challenge task was successful in causing an increase in the perception of stress in both depressed and control participants.

A comparison of perceived stress reactions between depressed and control participants shows that there were no significant differences between groups from baseline to the stressor ($t_{change1}$ (28) = 0.70; p = 0.49) or from the stressor to 60-minutes afterwards ($t_{change2}$ (28) = 0.26; p = 0.80.) This finding does not support the hypothesis that depressed participants would demonstrate significantly greater increases in perceived stress as compared to controls, but the data do support the hypothesis that depression is associated with elevated baseline levels of distress in a laboratory setting. These effects were supported by mixed-model analysis of variance, indicating a main effect for time (F (2, 27 = 18.18, p < 0.001) and for depression status (F (2, 27) = 19.25., p < 0.001), whereas no significant interaction term was observed (F2, 27 = 3.06. p = 0.09.) Detailed analyses of simple effects are shown in Table 7.

Table 7. Paired T-Tests of Perceived Stress Before, Immediately After, and 60 Minutes After the Mental Stress (MS) Challenge

	Control		Depressed	
	Pre-MS to MS (n = 16)	MS to Post -MS (n = 16)	Pre-MS To MS (n = 14)	MS to Post-MS (n = 14)
Paired T-Test	t (15) =-2.91 p=0.01	t(15) =4.27 p<0.01	t (13)=-2.90 p<0.01	t(13)=2.43 p=0.03

Changes in self-reported perceived stress following the laboratory stressor were correlated with changes in cortisol levels taken during the same time frame. Change scores were used to determine mood and cortisol reactivity. No significant relationship between perceived acute distress increases and cortisol reactivity ($r_{change\ \#1}$ = 0.24, p = 0.20) was found. The correlation between perceived distress levels at recovery from baseline and parallel changes in cortisol levels was also insignificant ($r_{change\ \#2}$ = 0.05, p = 0.80.)

Based on the regression model for mediation proposed by Barron and Kenney (1989), regression models were not constructed due to the lack of correlation between cortisol levels and perceived stress as measured by the PSS, Likert scales, and ambulatory diary. We also examined the correspondence between the three domains of distress, and found that the PSS scores correlated with 4 of the 5 diary stress levels but not with laboratory-based Likert change score.

In order to examine whether participant-reported perceived stress actually demonstrated the expected difference between depressed and control participants, comparison analyses were conducted on PSS scores, ambulatory perceived stress reports, and Likert-reported stress reactivity. There was no significant difference between groups on Likert reactivity from baseline to stressor ($F = 0.48$; $p = 0.49$) or from stressor to 60 minutes post ($F = 0.07$; $p = 0.79$.) There was a significant difference between groups on PSS scores ($F = 27.38$; $p < 0.01$), and on time spent at stress level 1 ($F = 6.34$; $p = 0.02$.) This suggests that differential reactivity between depressed and non-depressed individuals occurred only the measures of long-term perceived stress (the PSS inquired about the past week and the diary recorded 24 hours) and not uniformly on measures of acute perceived stress. This supposition is supported by the finding that PSS scores correlate with 4 of the 5 diary stress levels but not with either Likert change score.

Table 8. Correlations Between PSS Scores and Time Spent at Stress Levels

	Level 1	Level 2	Level 3	Level 4	Level 5
Correlation	$r = -0.62$	$r = 0.50$	$r = 0.48$	$r = 0.26$	$r = 0.47$
Significance	$p < 0.01$*	$p = 0.02$*	$p = 0.02$*	$p = 0.24$	$p = 0.03$*

Table 9. Correlations Between PSS Scores and Likert Change Scores

	Pre-MS to MS	MS to post-MS
Correlation	r = -0.13	r = 0.04
Significance	p = 0.51	p = 0.84

Discussion

This study hypothesized that the relationship between depression and elevated levels of cortisol established in previous studies would be mediated by heightened levels of perceived stress in depressed individuals. Instead, ambulatory cortisol levels examined in hypothesis #2 varied between groups independent of self-reported stress levels, suggesting that the effect of perceived stress on cortisol levels is not greater in depressed participants than in control participants. Finally, analysis of data in hypothesis #3 demonstrated that cortisol elevations associated with perceived stress occurred only in control participants.

A blunted cortisol response was, however, observed in depressed participants. While the difference between depressed and control participants was not statistically significant, a stronger response to waking was present in control participants as compared to depressed participants. This finding is not consistent with hypothesis #1, which theorized that depressed individuals would demonstrate a greater salivary cortisol response to waking as compared to control participants. It may, however, be consistent with a blunting of cortisol responses that has been indicated in several other studies (Morrison et al., 2001; Oldehinkel et al., 2001; Bremmer et al., 2006)

As previously discussed, depressed individuals demonstrate altered HPA axis function including increased secretion of cortisol from the adrenal cortex, impairment of the negative cortisol feedback loop, and hypertrophy of the adrenal gland (Halbreich, Asnis, Shindledecker, Zumoff, & Nathan, 1985; Pfohl, Sherman, Schlechte, & Winokur, 1985.) There is evidence that over time these physiological responses may result in blunted cortisol elevations in response to perceived stress (Burke, Davis, Otte, & Mohr, 2005.) This physiological blunting of the stress response has been demonstrated primarily in laboratory-based dexamethasone administration tests which utilize a synthetic glucocorticoid to mimic endogenous responses to psychological stressors. This evidence is limited, however, as dexamethasone administration is a model for glucocorticoid activity but fails to activate the full range of physiological responses to psychological stressors, including non-glucocorticoid HPA axis responses, mineralocorticoid responses, and limbic system responses (Burke, Davis, Otte, & Mohr, 2005.) Despite weaknesses associated with dexamethasone administration, this testing represents one model of the possible effects of long-term exposure to HPA axis activation that may explain the decreased cortisol responses observed in this study's depressed participants.

There is also evidence that HPA axis activity may vary between individuals; some studies have demonstrated significant variability in HPA response patterns among depressed individuals. HPA response patterns have been shown to vary by age and gender, stressor specifics such as duration and type (i.e., public speaking as opposed to cognitive tasks), depression subtype and severity, and the presence of early life stress (Burke, Davis, Otte, & Mohr, 2005.) The failure of this study to demonstrate the hypothesized differences between groups may be due to such differences among individuals within the study groups. For example, a recent study found that blunted cortisol responses were more common in depressed individuals who also demonstrated joint disease, a history of smoking, and female sex. Elevated cortisol responses were more common in depressed individuals demonstrating older age, cardiovascular disease, current use of NSAIDs, and male sex (Bremmer et al., 2006.) Exhaustion and professional burnout (Preussner, Hellhammer, & Kirschbaum, 1999) fatigue (Mommersteeg et al., 2006), and morning vs. evening preference for activity (Kudielka et al., 2006) have also been examined as potential individual influences on cortisol responses. While the present study accounted for some of these variables, they were not all measured and may have impacted individual responses to perceived stress.

In addition to blunted cortisol responses among depressed participants in the ambulatory setting, the present study found that both depressed and control participants displayed a decrease in salivary cortisol levels in response to stressors in the laboratory setting. Salivary cortisol levels changed significantly from baseline to 60 minutes after the stressor, but the direction of change did not support the hypothesis. Specifically, rather than demonstrating an increase in cortisol levels, both depressed and control participants displayed a decrease in salivary cortisol levels. There was no significant difference between depressed and control participants in change over time, suggesting that the effect of perceived stress on cortisol levels is not greater in depressed participants than in control participants. This observation may be explained in part by the relatively high baseline cortisol levels prior to the mental challenge task. Levels were significantly higher at the laboratory resting period (approximately 4.5 nmol/l) compared to the ambulatory measurement obtained prior to bed-time (approximately 1 nmol/l), whereas laboratory post-challenge 60 minute recovery levels (2.8 nmol/l) were closer to the ambulatory baseline measures.

The elevated baseline levels present in the laboratory portion of this study could be related to the time of day during which the measurements were taken. Burke et al. (2005) conducted a meta-analysis of studies concerning cortisol responses to psychological stressors and found that depressed participants demonstrated significantly higher baseline cortisol levels than control participants when studies were conducted in the afternoon. In contrast, depressed participants demonstrated lower baseline cortisol levels than non-depressed controls in the morning. The present study collected all laboratory cortisol samples in the afternoon between 12 and 4 PM. It may therefore be that the study protocol was incorrectly timed to detect statistically significant between-group differences in salivary cortisol responses.

Between-group differences in perceived stress were also examined in this study. Aim #3 examined the role of perceived stress in laboratory and ambulatory salivary cortisol increases across three domains of perceived stress: (a) retrospective self-report using the Perceived Stress Scale; (b) ambulatory self report of distress levels during 24 hours of monitoring; and (c) acute perceived stress response to a laboratory stressor. The relationship between (a) PSS scores and laboratory resting cortisol levels approached significance only in control participants, which was in contrast to hypothesis #3 which theorized that perceived stress would influence elevated cortisol levels in depressed individuals. Analysis of (b) ambulatory diary levels of perceived stress revealed that the only significant relationship between perceived stress and morning increases in cortisol was in control participants who reported their stress levels at 2 out of 5 on the Likert scale. Daily cortisol averages also demonstrated a significant correlation with perceived stress level 2 in control participants. There were no significant relationships with any other levels of perceived stress. Analysis of (c) Likert score elevations showed that there was no significant relationship between perceived stress increases and cortisol reactivity from the baseline measure to the stressor reading or from the stressor to 60 minutes after the stressor.

Overall, the role of perceived stress in laboratory and ambulatory salivary cortisol increases could not be confirmed in the present study. This may be due to the previously discussed individual differences in cortisol production. There is also limited evidence demonstrating an influence of sex and age on the relationship between negative affect and cortisol levels. In studies examining role of sex and age in this interaction, cortisol elevations associated with negative affect occurred only in men (Schaeffer & Baum, 1984.) or more significantly in elderly men (Kudielka et al., 2004.) Other studies have demonstrated that women are more likely to demonstrate stress vulnerability and to report subjective stress (Kudielka & Kirschbaum, 2005.) Although the study groups were matched for gender, a differential reactivity to perceived stress on the part of male or female participants could have impacted analysis.

Study Limitations

Power: The nonsignificant correlation between the target variables addressed in this study may be the result of small effect sizes. Based on previous research, the study was powered to detect an r of 0.47. However, demonstrated r-values in this study averaged 0.20. At an alpha level of 0.05 and power of 0.80, 190 participants would have been necessary to detect an effect size of 0.20. However, even with low power the pattern of results that this study did detect is such that the effect is not in the hypothesized direction, and this problem cannot be explained by power alone.

Compliance: Problems establishing relationships between depression and perceived stress with ambulatory-assessed or laboratory-based salivary cortisol levels may have been related to participant compliance and the nature of the laboratory challenge tasks. Non-compliance in ambulatory settings can take the form of non-adherence to the requested protocol with accurate reporting of the time a sample was taken, or non-adherence to the protocol with inaccurate reporting of the time a cortisol sample was taken. Inaccurate reporting time is more detrimental to the integrity of the study, because it results in increased errors in the data and alterations in expected diurnal cortisol patterns (Kudielka, Broderick, & Kirschbaum, 2003.) A recent study demonstrated that participant reports of sampling times were significantly inflated in order to make sampling behaviors appear more compliant with study protocols than they actually were (Broderick, Arnold, Kudielka, & Kirschbaum, 2003.) This study found that compliance with salivary cortisol sampling protocol cannot be assumed valid in the absence of independent monitoring. While the present study did provide participants with instructions to record actual sampling times using the ambulatory diary, there was no objective method for establishing sampling time independent of participant report and only 7 of the total 22 participants recorded their sampling times as requested. This may have resulted in inaccurate reporting of the time each saliva sample was actually taken, which would have had a significant impact during comparison analyses conducted in this study. The sampling protocol used in this study specifically requested that participants record the exact time of saliva sampling using the ambulatory diary. Interestingly, while 6 control participants complied with this request, only one depressed participant did so. This suggests that compliance with study protocol may be a particular problem in studies that incorporate participants with impaired motivation, energy levels, or attention.

Stressor Tasks: Problems with establishing relationships in the laboratory setting may have been related to establishing appropriate levels of perceived stress, and the timing and precision of the salivary-based assays. An analysis of perceived stress reports demonstrated that there were significant differences in perceived stress from baseline to the onset of the stressor, and from the onset to 60-minutes after the offset in both the depressed and the control groups. This demonstrates that the mental challenge task was successful in causing an increase in the perception of stress in both depressed and control participants. It is possible, however, that the stressor was not sufficient to generate large increases in perceived stress in the non-depressed control group. If the non-depressed controls were not stressed by the laboratory challenge, comparison of the depressed and control groups would not have generated results that were indicative of true differences in responsivity.

Duration of Stressor: It is also possible that significant differences in perceived stress and resulting cortisol changes occur only in response to chronic stress and not in response to brief stressors, such as the laboratory task. This possibility is supported by the finding that the depression and control groups demonstrated significant differences on reports of 24 hour stress and stress over the past week, but not on measures of reactivity that spanned 60 minutes. This finding is also supported by limited literature demonstrating that *trait* negative affect (a mean affect level over multiple days of assessment) is associated with elevated cortisol levels, but *state* negative affect (deviations from a mean affect level on a specific day) is not (Polk et al., 2005.)

Implications

This study aimed to establish the role of perceived stress in the relationship between depression and increases in cortisol production. This may be related to the influence of individual differences related to sex, age, exhaustion, professional burnout, or preferences for morning vs. evening activities. Significant differences in perceived stress between groups occurred only on the measures of long-term ambulatory and daily-life stress, and were not present following laboratory-induced stress. This finding could be related to the time of day during which ambulatory measures were taken, or to problems with participant compliance. There is also some support for the idea that only perceived stress resulting from repeated, or chronic, stress experiences significantly influences physiological response in depressed individuals. This possibility is supported by research demonstrating the association of global levels of sustained stress, referred to as allostatic load, with changes in HPA axis function (McEwan, 1998; Sterling and Eyer, 1988.)

The possible influence of trait vs. state negative affect, individual differences, and participant compliance suggests that the methodology of the current study did not address several possible influences on cortisol deviations. It is also possible that perceived stress is not a factor in the relationship between depression and HPA axis alterations. Several studies have found that previous findings identifying perceived stress as a construct related to cortisol deviations incorporate measurement of additional relevant variables such as fatigue (Mommersteeg et al., 2006) or coping strategies (Folkman et al., 1986.) It has been suggested that these variables, rather than perceived stress, are the actual influences on HPA axis alterations.

Although this study was unable to establish a relationship between perceived stress and cortisol deviations in depressed individuals, depressed participants did demonstrate a flattened circadian rhythm in analysis of diurnal cortisol production. This is similar to previous research demonstrating that repeated or sustained HPA axis activation may result in blunted cortisol elevations in response to perceived stress (Burke, Davis, Otte, & Mohr, 2005.) The present study also found significant differences in perceived stress between groups in measures of long-term ambulatory stress which were not present following short-term laboratory-induced stress. Combined with the findings demonstrating a flattened circadian rhythm, the difference in long-term vs. short-term findings might indicate that long-term exposure to perceived stress is related to HPA axis "burnout", which could generate blunted cortisol elevations in depressed individuals.

Future directions

Additional research is needed to establish the relationship between perceived levels of stress resulting from repeated, or chronic, stress experiences and HPA axis responses in depressed individuals. Future research should clarify the possible role of time of day on cortisol production (Burke et al., 2005.) Additionally, clarification is needed regarding the influence of age and sex (Schaeffer & Baum, 1984; Kudielka et al., 2004) on cortisol deviations.

The physiological consequences of increased reactivity to perceived stress could best be examined in a longitudinal study. The current study found some evidence that long-term perceived stress is more likely to be related to changes in HPA axis function than acute stress perception. A longitudinal format would provide the opportunity to examine exposure to long-term perceived stress and to establish whether depressed individuals demonstrating increased reactivity develop health problems, including cardiovascular disease, cancer, and all-cause mortality. It would also provide additional time with study participants during which measurement of additional variables such as exhaustion, burnout, and morning vs. evening preference could be accomplished.

Clinically, interventions aimed at decreasing the health impacts of HPA axis alterations in depressed individuals could be developed based on the available research findings. Educating individuals regarding the possible role of perceived stress, age, and gender might enable depressed individuals to make life changes that impact disease development or progression. If future longitudinal research clarifies the role of perceived stress in cortisol deviations, specific recommendations could be made regarding exposure to stress, or alternately regarding the importance of other variables such as exhaustion and professional burnout (Preussner, Hellhammer, & Kirschbaum, 1999) or fatigue (Mommersteeg et al.)

Tables

Table 1. Salivary Cortisol at Bedtime and at Waking

	Control		Depressed	
	Bedtime n = 11	Waking n = 11	Bedtime n = 12	Waking n = 12
Salivary Cortisol (nmol/l)	0.77 (±0.66)	12.37 (±4.95)	1.87 (±4.16)	5.75 (±3.12)

Table 2. Salivary Cortisol Before, Immediately After, and 60 Minutes After the Mental Stress (MS) Challenge.

	Control N = 16			Depressed N = 12		
	Before MS	After MS	60 Minutes	Before MS	After MS	60 Minutes
Salivary Cortisol (nmol/l)	4.00 ±2.08	4.19 ±2.58	2.63 ±1.24	5.39 ±4.52	4.71 ±4.07	3.06 ±2.07

Table 3. Perceived Stress Before, Immediately After, and 60 Minutes After the Mental Stress (MS) Challenge

	Control N = 16			Depressed N = 14		
	Before MS	After MS	60 Minutes	Before MS	After MS	60 Minutes
Perceived Stress (Mean Level)	1.56	2.94	1.25	3.29	5.14	3.64

Table 4. Ambulatory Distress and Depression Group Status

	Relationship to Depression Group Status	
	F	p
Distress Level		
1	6.34	0.02*
2	1.42	0.25
3	3.51	0.77
4	0.05	0.83
5	1.96	0.18
Sleep	0.83	0.37
Missing	6.68	0.02

Table 5. Diary Stress Levels and Cortisol Awakening Responses

	Total N= 22		Control N=11		Depressed N= 11	
	r	p	r	p	r	p
Distress Level						
1	0.06	0.80	-0.44	0.18	0.32	0.37
2	0.08	0.73	0.78	<0.01*	0.21	0.55
3	-0.01	0.95	0.48	0.14	0.24	0.51
4	-0.14	0.53	0.05	0.90	-0.59	0.07
5	-0.27	0.23	none	none	0.01	0.97
Sleep	-0.08	0.73	-0.11	0.75	-0.16	0.64
Missing	0.06	0.81	0.23	0.50	0.26	0.34

Table 6. Diary Stress Levels and Cortisol Daily Averages

	Total N = 22	Control N = 11		Depressed N = 11	
	mean (s.d.)	r	p	r	p
Distress Level					
1	461.45 (357.04)	-0.37	0.29	-0.14	0.73
2	122.68 (148.85)	0.66	0.04*	-0.00	0.99
3	123.18 (159.19)	0.47	0.17	0.32	0.39
4	62.00 (131.78)	0.08	0.84	0.38	0.31
5	4.09 (14.03)	none	none	-0.37	0.32
Sleep	461.45 (357.04)	-0.37	0.29	-0.12	0.76
Missing	122.68 (148.85)	0.34	0.34	-0.78	0.85

Table 7. Paired T-Tests of Perceived Stress Before, Immediately After, and 60 Minutes After the Mental Stress (MS) Challenge

	Control		Depressed	
	Pre-MS to MS (n = 16)	MS to Post -MS (n = 16)	Pre-MS To MS (n = 14)	MS to Post-MS (n = 14)
Paired T-Test	t(15) =-2.91 p=0.01	t(15) =4.27 p<0.01	t(13)=-2.90 p<0.01	t(13)=2.43 p=0.03

Table 8. Correlations Between PSS Scores and Time Spent at Stress Levels

	Level 1	Level 2	Level 3	Level 4	Level 5
Correlation	r = -0.62	r = 0.50	r = 0.48	r = 0.26	r = 0.47
Significance	p <0.01*	p = 0.02*	p = 0.02*	p = 0.24	p = 0.03*

Table 9. Correlations Between PSS Scores and Likert Change Scores

	Pre-MS to MS	MS to post-MS
Correlation	r = -0.13	r = 0.04
Significance	p = 0.51	p = 0.84

Figure 1. Theoretical Model of Experiment

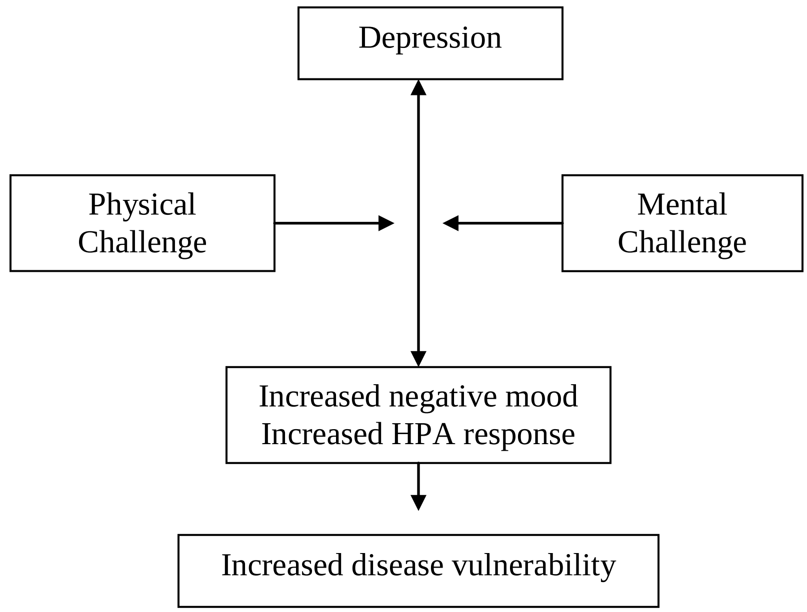


Figure 2. Laboratory Procedures

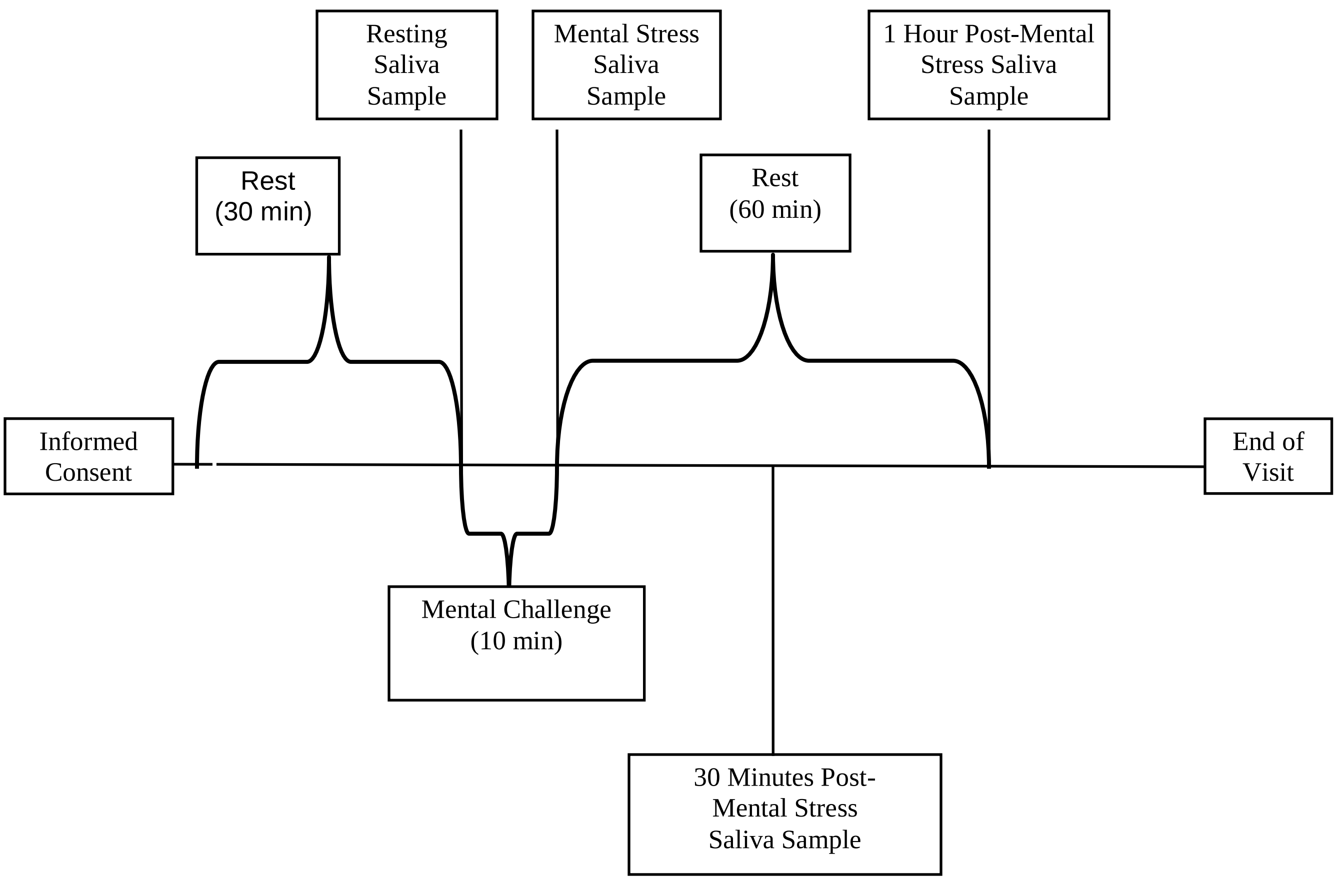


Figure 3. Ambulatory Procedure

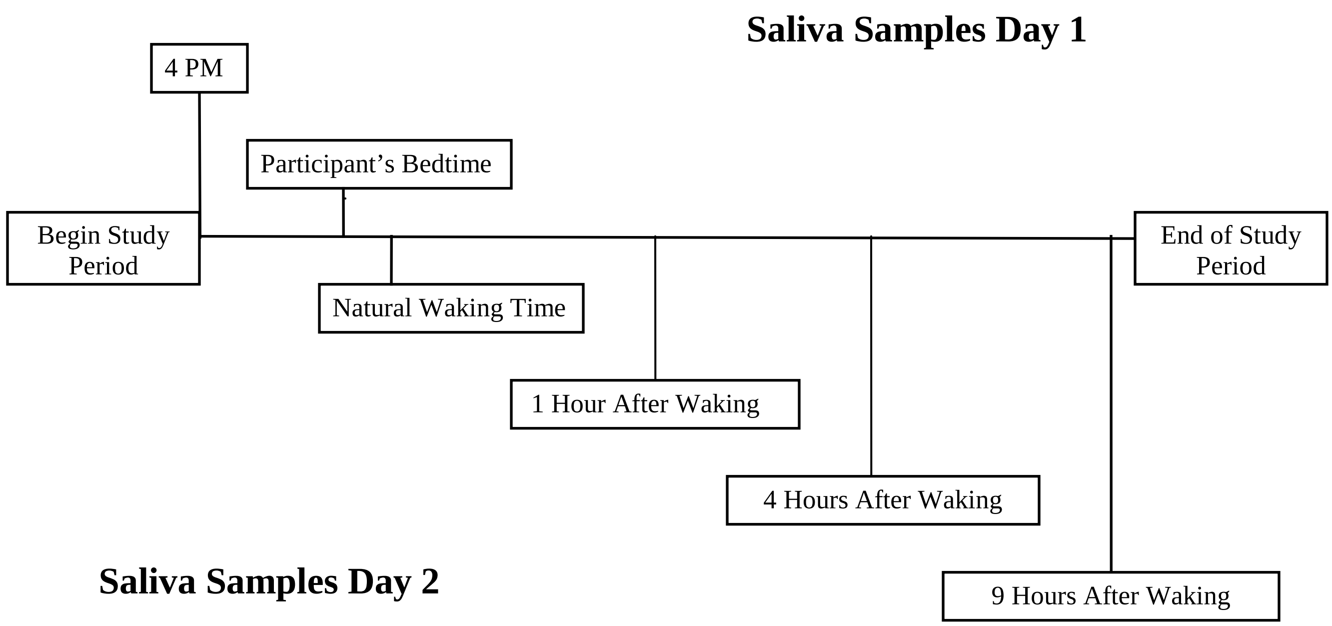
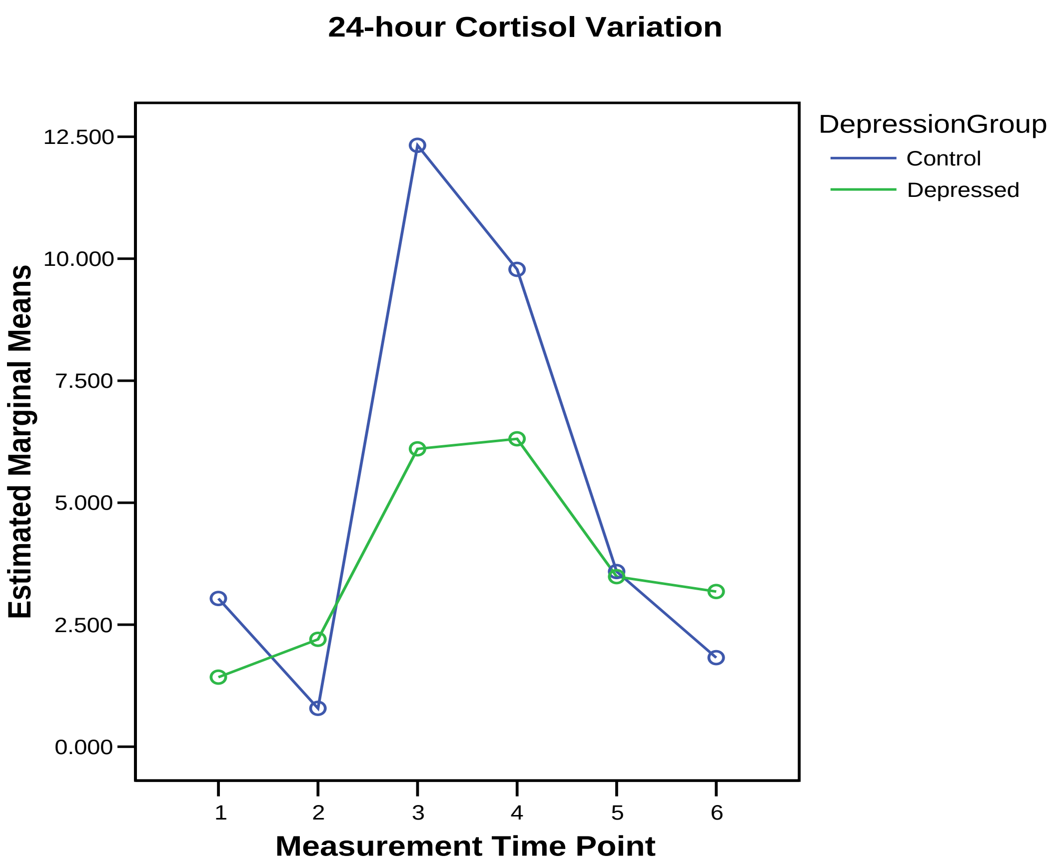


Figure 4. 24-hour Cortisol Variation



References

American Heart Association (2006.) Coronary heart disease, acute coronary syndrome, and angina pectoris. American Heart Association [On-line]. Available: http://www.americanheart.org/statistics

Blumenthal, J. A., Burg, M. M., Barefoot, J., Williams, R. B., Haney, T., & Zimet, G. (1987.) Social support, type A behavior, and coronary artery disease. *Psychosom.Med., 49,* 331-340.

Blumenthal, J. A., Jiang, W., Waugh, R. A., Frid, D. J., Morris, J. J., Coleman, R. E. et al. (1995.) Mental stress-induced ischemia in the laboratory and ambulatory ischemia during daily life. Association and hemodynamic features. *Circulation, 92,* 2102-2108.

Bremmer, M. A., Deeg, D. J., Beekman, A. T., Penninx, B. W., Lips, P., & Hoogendijk, W. J. (2007.) Major Depression in Late Life Is Associated with Both Hypo- and Hypercortisolemia. *Biol.Psychiatry, 11,* 1-8.

Broderick, J. E., Arnold, D., Kudielka, B. M., & Kirschbaum, C. (2004.) Salivary cortisol sampling compliance: comparison of patients and healthy volunteers. *Psychoneuroendocrinology, 29,* 636-650.

Brunner, E. J., Hemingway, H., Walker, B. R., Page, M., Clarke, P., Juneja, M. et al. (2002.) Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: nested case-control study. *Circulation, 106,* 2659-2665.

Burke, H., Davis, M., Otte, C., & Mohr, D. (2005.) Depression and cortisol responses to psychological stress: A meta-analysis. *Psychoneuroendocrinology, 30,* 846-856.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H. et al. (2003.) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science, 301,* 386-389.

Chrousos, G. P. & Gold, P. W. (1992.) The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA, 267,* 1244-1252.

Clara, I. P., Cox, B. J., Enns, M. W., Murray, L. T., & Torgrudc, L. J. (2003.) Confirmatory factor analysis of the multidimensional scale of perceived social support in clinically distressed and student samples. *J.Pers.Assess., 81,* 265-270.

Cohen, L. H., Gunthert, K. C., Butler, A. C., O'Neill, S. C., & Tolpin, L. H. (2005.) Daily affective reactivity as a prospective predictor of depressive symptoms. *J.Pers., 73,* 1687-1713.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983.) A global measure of perceived stress. *J.Health Soc.Behav., 24,* 385-396.

Cohen, S., Kessler, R., & Gordon, L. (1997.) **Strategies for measuring stress in studies of psychiatric and physical disorders**. In S.Cohen, R. Kessler, & L. Gordon

(Eds.), *Measuring stress: A guide for health and social scientists* (pp. 3-26.) New York.

Corsini, R. (2002.) *The Dictionary of Psychology*. New York: Brunner-Routledge.

Dahlem, N. W., Zimet, G. D., & Walker, R. R. (1991.) The Multidimensional Scale of Perceived Social Support: a confirmation study. *J.Clin.Psychol., 47,* 756-761.

Deuster, P. A., Petrides, J. S., Singh, A., Chrousos, G. P., & Poth, M. (2000.) Endocrine response to high-intensity exercise: dose-dependent effects of dexamethasone. *J.Clin.Endocrinol.Metab, 85,* 1066-1073.

Dhillo, W. S., Kong, W. M., Le Roux, C. W., aghband-Zadeh, J., Jones, J., Carter, G. et al. (2002.) Cortisol-binding globulin is important in the interpretation of dynamic tests of the hypothalamic--pituitary--adrenal axis. *Eur.J.Endocrinol., 146*, 231-235.

Dougall, A. & Baum, A. (1991.) Stress, Health, and Illness. In A.Baum, T.Revenson, & J.Singer (Eds.), *Handbook of Health Psychology* (pp. 321-337.) New Jersey: Lawrence Erlbaum Associates, Inc.

Feldman, P. J., Cohen, S., Lepore, S. J., Matthews, K. A., Kamarck, T. W., & Marsland, A. L. (1999.) Negative emotions and acute physiological responses to stress. *Ann.Behav.Med., 21,* 216-222.

Gold, P. W., Gabry, K. E., Yasuda, M. R., & Chrousos, G. P. (2002.) Divergent endocrine abnormalities in melancholic and atypical depression: clinical and pathophysiologic implications. *Endocrinol.Metab Clin.North Am., 31,* 37-62, vi.

Gottdiener, J. S., Krantz, D. S., Howell, R. H., Hecht, G. M., Klein, J., Falconer, J. J. et al. (1994.) Induction of silent myocardial ischemia with mental stress testing: relation to the triggers of ischemia during daily life activities and to ischemic functional severity. *J.Am.Coll.Cardiol., 24,* 1645-1651.

Gozansky, W. S., Lynn, J. S., Laudenslager, M. L., & Kohrt, W. M. (2005.) Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic--pituitary--adrenal axis activity. *Clin.Endocrinol.(Oxf), 63,* 336-341.

Greenberg, P. E., Kessler, R. C., Birnbaum, H. G., Leong, S. A., Lowe, S. W., Berglund, P. A. et al. (2003.) The economic burden of depression in the United States: how did it change between 1990 and 2000? *J.Clin.Psychiatry, 64*, 1465-1475.

Gunthert, K., Butler, A., & Cohen, L. (2005.) Predictive role of daily coping and affective reactivity in cognitive therapy outcome: Application of a daily process design to psychotherapy research. *Behavior Therapy, 36,* 79-90.

Guyton, A. & Hall, J. (2000.) *Textbook of Medical Psychology*. Philadelphia: Saunders.

Halbreich, U., Asnis, G. M., Shindledecker, R., Zumoff, B., & Nathan, R. S. (1985.) Cortisol secretion in endogenous depression. I. Basal plasma levels. *Arch.Gen.Psychiatry, 42,* 904-908.

Hamilton, M. (1967.) Development of a rating scale for primary depressive illness. *British Journal of Social and Clinical Psychology, 6,* 278-296.

Hedlund, J. & Virmani, R. (1979.) The Hamilton rating scale for depression: A comprehensive review. *Journal of Operational Psychiatry, 11,* 48-65.

Hjemdahl, P. (2002.) Stress and the metabolic syndrome: an interesting but enigmatic association. *Circulation, 106,* 2634-2636.

Hooley, J. M., Orley, J., & Teasdale, J. D. (1986.) Levels of expressed emotion and relapse in depressed patients. *Br.J.Psychiatry, 148,* 642-647.

Ironson, G., Taylor, C. B., Boltwood, M., Bartzokis, T., Dennis, C., Chesney, M. et al. (1992.) Effects of anger on left ventricular ejection fraction in coronary artery disease. *Am.J.Cardiol., 70,* 281-285.

Jern, C., Manhem, K., Eriksson, E., Tengborn, L., Risberg, B., & Jern, S. (1991.) Hemostatic responses to mental stress during the menstrual cycle. *Thromb.Haemost., 66,* 614-618.

Johnson, J., Weissman, M. M., & Klerman, G. L. (1992.) Service utilization and social morbidity associated with depressive symptoms in the community. *JAMA, 267,* 1478-1483.

Joiner, T. E., Jr., Steer, R. A., Beck, A. T., Schmidt, N. B., Rudd, M. D., & Catanzaro, S. J. (1999.) Physiological hyperarousal: construct validity of a central aspect of the tripartite model of depression and anxiety. *J.Abnorm.Psychol., 108,* 290-298.

Kaplan, J. R., Pettersson, K., Manuck, S. B., & Olsson, G. (1991.) Role of sympathoadrenal medullary activation in the initiation and progression of atherosclerosis. *Circulation, 84,* VI23-VI32.

Kessler, R. C., McGonagle, K. A., Zhao, S., Nelson, C. B., Hughes, M., Eshleman, S. et al. (1994.) Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch.Gen.Psychiatry, 51,* 8-19.

Kessler, R. C. (1997.) The effects of stressful life events on depression. *Annu.Rev.Psychol., 48,* 191-214.

Kimmel, P. L., Peterson, R. A., Weihs, K. L., Simmens, S. J., Alleyne, S., Cruz, I. et al. (2000.) Multiple measurements of depression predict mortality in a longitudinal study of chronic hemodialysis outpatients. *Kidney Int., 57,* 2093-2098.

Kirschbaum, C. & Hellhammer, D. H. (1989.) Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology, 22,* 150-169.

Kop, W. J., Krantz, D. S., Nearing, B. D., Gottdiener, J. S., Quigley, J. F., O'Callahan, M. et al. (2004.) Effects of acute mental stress and exercise on T-wave alternans in patients with implantable cardioverter defibrillators and controls. *Circulation, 109,* 1864-1869.

Kovacs, M. & Beck, A. T. (1978.) Maladaptive cognitive structures in depression. *Am.J.Psychiatry, 135,* 525-533.

Krantz, D. & Falconer, J. (1997.) Measurement of Cardiovascular Responses. In S.Cohen, R. Kessler, & L. Gordon (Eds.), *Measuring stress: A guide for health and social scientists* (pp. 193-212.) New York: Oxford University Press.

Krantz, D. S., Kop, W. J., Santiago, H. T., & Gottdiener, J. S. (1996.) Mental stress as a trigger of myocardial ischemia and infarction. *Cardiol.Clin., 14,* 271-287.

Krantz, D. S., Kop, W. J., Gabbay, F. H., Rozanski, A., Barnard, M., Klein, J. et al. (1996.) Circadian variation of ambulatory myocardial ischemia. Triggering by daily activities and evidence for an endogenous circadian component. *Circulation, 93,* 1364-1371.

Krantz, D. S., Kop, W. J., Santiago, H. T., & Gottdiener, J. S. (1996.) Mental stress as a trigger of myocardial ischemia and infarction. *Cardiol.Clin., 14,* 271-287.

Kudielka, B., Federenko, I., Hellhammer, D., & Wust, S. (2006.) Morningness and eveningness: the free cortisol rise after awakening in “early birds” and “night owls”. *Biological Psychology, 72,* 141-146.

Kudielka, B., & Kirschbaum, C. (2005.) Sex differences in HPA axis responses to stress: a review. *Biol.Psychol., 69,* 113-132.

Kunz-Ebrecht, S. R., Mohamed-Ali, V., Feldman, P. J., Kirschbaum, C., & Steptoe, A. (2003.) Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines. *Brain Behav.Immun., 17,* 373-383.

Lazarus, R. & Folkman, S. (1984.) *Stress, appraisal, and coping*. New York: Springer.

Malla, A. K., Cortese, L., Shaw, T. S., & Ginsberg, B. (1990.) Life events and relapse in schizophrenia. A one year prospective study. *Soc.Psychiatry Psychiatr.Epidemiol., 25,* 221-224.

McEwen, B. S. (2006.) Protective and damaging effects of stress mediators: central role of the brain. *Dialogues.Clin.Neurosci., 8,* 367-381.

McNair, D., Lorr, M., & Droppelman, L. (1992.) *Manual for the Profile of Mood States*. San Diego: Education and Industrial Testing Service.

Mommersteeg, P., Keijers, G., Heijnen, C., Verbraak, M., & Doornen, L. (2006). Cortisol deviations in people with burnout before and after psychotherapy: A pilot study. *Health Psychology, 25,* 243-248.

Monroe, S. M. (1983.) Social support and disorder: toward an untangling of cause and effect. *Am.J.Community Psychol., 11*, 81-97.

Monroe, S. M. & Kelley, J. (1997.) Measurement of Stress Appraisal. In L.Cohen, R. Kessler, & L. Gordon (Eds.), *Measuring stress: A guide for health and social scientists* (pp. 122-147.) New York: Oxford University Press.

Morrison, M., Redei, E., TenHave, T., Parmelee, P., Boyce, A., Sinha, P., et al. (2001). Dehydroepiandrosterone sulfate and psychiatric measures in a frail, elderly residential care population. *Biol Psychiatry, 47,* 144-150.

Myin-Germeys, I., Van, O. J., Schwartz, J. E., Stone, A. A., & Delespaul, P. A. (2001.) Emotional reactivity to daily life stress in psychosis. *Arch.Gen.Psychiatry, 58,* 1137-1144.

Myin-Germeys, I., Peeters, F., Havermans, R., Nicolson, N. A., DeVries, M. W., Delespaul, P. et al. (2003.) Emotional reactivity to daily life stress in psychosis and affective disorder: an experience sampling study. *Acta Psychiatr.Scand., 107,* 124-131.

Oldehinkel, A., Van, B., Flentgt, F., Bouhuys, A., Horst, G., & Ormel, J. (2001). Urinary free cortisol excretion in elderly persons with minor and major depression. *Psychiatry Res, 104,* 39-47.

Oltras, C. M., Mora, F., & Vives, F. (1987.) Beta-endorphin and ACTH in plasma: effects of physical and psychological stress. *Life Sci., 40,* 1683-1686.

Ormel, J., VonKorff, M., Ustun, T. B., Pini, S., Korten, A., & Oldehinkel, T. (1994.) Common mental disorders and disability across cultures. Results from the WHO Collaborative Study on Psychological Problems in General Health Care. *JAMA, 272,* 1741-1748.

Patterson, S. M., Gottdiener, J. S., Hecht, G., Vargot, S., & Krantz, D. S. (1993.) Effects of acute mental stress on serum lipids: mediating effects of plasma volume. *Psychosom.Med., 55,* 525-532.

Pfohl, B., Sherman, B., Schlechte, J., & Winokur, G. (1985.) Differences in plasma ACTH and cortisol between depressed patients and normal controls. *Biol.Psychiatry, 20,* 1055-1072.

Polk, D., Cohen, S., Doyle, W., Skoner, D., & Kirschbaum, C. (2005.) State and trait affect as predictors of salivary cortisol in healthy adults. *Psychoneuroendocrinology, 30,* 261-272.

Pruessner, J., Hellhammer, D., & Kirschbaum, C. (1999.) Burnout, perceived stress, and cortisol responses to awakening. *Psychosom.Med., 61,* 197-204.

Ramasubbu, R. & Patten, S. B. (2003.) Effect of depression on stroke morbidity and mortality. *Can.J.Psychiatry, 48,* 250-257.

Ranjit, N., Young, E. A., Raghunathan, T. E., & Kaplan, G. A. (2005.) Modeling cortisol rhythms in a population-based study. *Psychoneuroendocrinology, 30*, 615-624.

Rosengren, A., Hawken, S., Ounpuu, S., Sliwa, K., Zubaid, M., Almahmeed, W. A. et al. (2004.) Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet, 364,* 953-962.

Rozanski, A., Bairey, C. N., Krantz, D. S., Friedman, J., Resser, K. J., Morell, M. et al. (1988.) Mental stress and the induction of silent myocardial ischemia in patients with coronary artery disease. *N.Engl.J.Med., 318,* 1005-1012.

Schaeffer, M., & Baum, A. (1984). Adrenal cortical response to stress at Three Mile Island. *Psychosomatic Medicine, 46,* 227-237.

Scheier, M. F., Carver, C. S., & Bridges, M. W. (1994.) Distinguishing optimism from neuroticism (and trait anxiety, self-mastery, and self-esteem): a reevaluation of the Life Orientation Test. *J.Pers.Soc.Psychol., 67,* 1063-1078.

Selye, H. (1936.) A syndrome produced by diverse nocuous agents. *Nature, 138,* 32.

Selye, H. (1950.) *The physiology and pathology of exposure to stress*. Montreal: Acta, Inc.

Selye, H. (1975.) Confusion and controversy in the stress field. *Journal of Human Stress, 1,* 37-44.

Smith, G. D., Ben-Shlomo, Y., Beswick, A., Yarnell, J., Lightman, S., & Elwood, P. (2005.) Cortisol, testosterone, and coronary heart disease: prospective evidence from the Caerphilly study. *Circulation, 112,* 332-340.

Spitzer, R. L., Williams, J. B., Kroenke, K., Linzer, M., deGruy, F. V., III, Hahn, S. R. et al. (1994.) Utility of a new procedure for diagnosing mental disorders in primary care. The PRIME-MD 1000 study. *JAMA, 272,* 1749-1756.

Pruessner, J., Hellhammer, D., & Kirschbaum, C. (1999). Burnout, perceived stress, and cortisol responses to awakening. *Psychosomatic Medicine, 61,* 197-204.

Steele, A. & Wade, T. D. (2004.) The contribution of optimism and quality of life to depression in an acute coronary syndrome population. *Eur.J.Cardiovasc.Nurs., 3,* 231-237.

Sterling, P. & Eyer, J. (1988.) **Allostasis: A New Paradigm to Explain Arousal Pathology**. In S.Fischer & J. Reason (Eds.), *Handbook of Life Stress, Cognition and Health* (pp. 629-649.) New York: John Wiley & Sons.

Stone, A. (1997.) **Measurement of affective response**. In S.Cohen, R. Kessler, & L. Gordon (Eds.), *Measuring stress: A guide for health and social scientists* (pp. 148-171.)

Strohle, A. & Holsboer, F. (2003.) Stress responsive neurohormones in depression and anxiety. *Pharmacopsychiatry, 36 Suppl 3*, S207-S214.

Turner, R. & Wheaton, B. (1997.) **Checklist measurement of stressful life events**. In S.Cohen, R. Kessler, & L. Gordon (Eds.), *Measuring stress: A guide for health and social scientists.* (pp. 29-58.) New York: Oxford University Press.

Valente, S. M. & Saunders, J. M. (1997.) Diagnosis and treatment of major depression among people with cancer. *Cancer Nurs., 20,* 168-177.

van der, P. G., Bernards, N., Meijman, T. F., & Heijnen, C. J. (1999.) The effect of depressive symptomatology on plasma cortisol responses to acute bicycle exercise among post-menopausal women. *Psychiatry Res., 85,* 113-117.

von, K. R., Mills, P. J., Fainman, C., & Dimsdale, J. E. (2001.) Effects of psychological stress and psychiatric disorders on blood coagulation and fibrinolysis: a biobehavioral pathway to coronary artery disease? *Psychosom.Med., 63,* 531-544.

Wethington, E., Brown, G., & Kessler, R. (1997.) **Interview measurement of stressful life events**. In S.Cohen, R. Kessler, & L. Gordon (Eds.), *Measuring stress: A guide for health and social scientists* (pp. 59-79.)

Wulsin, L. R., Vaillant, G. E., & Wells, V. E. (1999.) A systematic review of the mortality of depression. *Psychosom.Med., 61,* 6-17.

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

Name ____

Date _________ Age ________ Gender (*Circle*): **M F** Other____________________

0 = Never	**1 = Almost Never**	**2 = Sometimes**	**3 = Fairly Often**	**4 = Very Often**

1. In the last month, how often have you been upset because of something that happened unexpectedly?	**0**	**1**	**2**	**3**	**4**
2. In the last month, how often have you felt that you were unable to control the important things in your life?..	**0**	**1**	**2**	**3**	**4**
3. In the last month, how often have you felt nervous and "stressed"?	**0**	**1**	**2**	**3**	**4**
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	**0**	**1**	**2**	**3**	**4**
5. In the last month, how often have you felt that things were going your way?...	**0**	**1**	**2**	**3**	**4**
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	**0**	**1**	**2**	**3**	**4**
7. In the last month, how often have you been able to control irritations in your life?...	**0**	**1**	**2**	**3**	**4**
8. In the last month, how often have you felt that you were on top of things?...	**0**	**1**	**2**	**3**	**4**
9. In the last month, how often have you been angered because of things that were outside of your control?	**0**	**1**	**2**	**3**	**4**
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?..........................	**0**	**1**	**2**	**3**	**4**

Likerts

Instructions:"I will now mention a list of words which describe feelings that people may have. Please listen carefully and rate, on a scale of 1 to 7, how much you have had that feeling during the previous task. 1 means "not at all" and 7 means "very much." So, the more you have a certain feeling, the higher the number will be."

	Not at all						Very much
worn-out:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
angry:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
energetic:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
challenged:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
fatigued:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
anxious:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
lively:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
interested:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
cheerful:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
annoyed:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
weary:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
bitter:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
vigorous:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
depressed:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
irritated:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
tired:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
in control:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
stressed:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
grouchy:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
frustrated:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
exhausted:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
like a failure:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
full of pep:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
peeved:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
interested:	(1)	(2)	(3)	(4)	(5)	(6)	(7)

Ambulatory Diary Page

START TIME___:___ AM / PM

POSTURE:
sit / stand / lying down

LOCATION:
home / work / other

BEEPED? yes / no

Tobacco: yes / no

Caffeine: yes / no

Alcohol: yes / no

ACTIVITY

going to sleep
sleeping
rest
washing/dressing
urinating/defecating
driving/passenger
shopping
eating/drinking
house activity
walking
up/down stairs
sexual activity
talking/listening
reading
clerical work
TV/radio
thinking/concentrating
waiting
other:____________

END TIME___:___ AM / PM

AMOUNT OF EFFORT

	Not at all				Very much
physical	1	2	3	4	5
mental	1	2	3	4	5

MOOD

frustrated	1	2	3	4	5
tense	1	2	3	4	5
sad	1	2	3	4	5
happy	1	2	3	4	5
stressed	1	2	3	4	5
tired	1	2	3	4	5

HOW MUCH DO YOU FEEL...

chest pain	1	2	3	4	5
short of breath	1	2	3	4	5

CIRCLE ONE: alone / with others